DOCKET NO.: CHIR-0158 (0316.005) **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: **Covacci *et al.***

Serial No.: **09/~~306,~~934** (360) Group Art Unit: **1645**

Filed: **July 26, 1999** Examiner: **P. Bui**

For: **HELICOBACTER PYLORI CYTOTOXIN PROTEINS USEFUL FOR VACCINES AND DIAGNOSTICS**

I, **Robin S. Quartin**, Registration No. **45,028** certify that this correspondence is being deposited with the U.S. Postal Service as First Class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.
On August 3, 2000

Robin S. Quartin Reg. No. **45,028**

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

I, Giuseppe Del Giudice, do hereby declare as follows:

1. I am a Research Director employed by Chiron SpA, in Siena, Italy.

2. I am a medical doctor (M.D.) with 15 years of experience in vaccine development.

3. I have read U.S. application serial number 09/306,934, filed July 26, 1999, and entitled "*Helicobacter pylori* cytotoxin proteins useful for vaccines and diagnostics" ("'934

application"). While I am not an inventor of the subject matter of the '934 application, I am quite familiar with the invention and the technology at issue. The '934 application claims priority to PCT/EP93/00472 (filed March 2, 1993) and PCT/EP93/00158 (filed January 25, 1993), which two PCT applications claimed priority benefit of Italian application Serial No. FI92A000052 (filed March 2, 1992).

4. The invention provides purified, recombinantly produced polypeptides of the *Helicobacter pylori* cytotoxin (VacA) protein[1], for use, among others, in vaccines. The component VacA polypeptides of such vaccines should induce an immune response, should not be toxic, and preferably, should protect against subsequent challenge by the pathogen *H. pylori.*

5. I have read the Official Action dated February 7, 2000 ("Action").

6. In the Action, the Examiner rejected claims 40 - 50 as allegedly being indefinite because of the use of the term "substantially," which the Examiner asserts is a relative term lacking comparative basis. I respectfully disagree.

7. The term "substantially" is used in the amended and original claims in conjunction with terms relating to toxicity in such phrases as "exhibits substantially no toxicity, or substantially reduced toxicity," and "exhibits no functional contribution to toxicity, or a substantially reduced functional contribution to toxicity." These phrases are used to describe the characteristics of the claimed *H. pylori* VacA polypeptides, and the *H. pylori* VacA polypeptide components of the claimed vaccines of the invention. The term "substantially," as it is used in the '934 application,

[1]In the specification of the '934 application, *H. pylori* cytotoxin is referred to as "CT." However, current terminology for the "vacuolating" cytotoxin protein of *H. pylori* is "VacA."

would have been, and is clearly understood by those of ordinary skill in the art, to mean that such *H. pylori* proteins, or fragments thereof, do not exhibit statistically significant cytotoxic effects and, thus, would be acceptable for use in human vaccines. Cytotoxicity can be routinely assessed in a variety of assays known to those of skill in the art, such as *in vitro* vacuolation of cells or cell lines, *e.g.* HeLa cells, and *in vivo* administration of purified VacA to mice to analyze gastric tissue damage.

8. In the Action, the Examiner rejected claims 40 - 50, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with the claims. The Examiner asserted that undue experimentation would have been required to determine which VacA (CT) polypeptides/proteins are effective for use as vaccines and which fragments of cytotoxin protein would "exhibit substantially no contribution to toxicity" or would be effective as components in a "prophylactic or therapeutic vaccine," at the time of filing. I respectfully disagree.

9. Chemically inactivated and genetically detoxified toxins were known to those of skill in the art, prior to March 2, 1992. For example, Exhibit A, which is a copy of Manetti *et al.*, 1995, *Infect. Immun.* 63:4476-4480, cites to prior studies (Pizza *et al.*, 1989) of chemically and genetically detoxified subunits of pertussis toxin, which also were capable of inducing protective immunity.

10. It is routine to determine, and was routine to determine, as of March 2, 1992, which polypeptide fragments of *H. pylori* VacA would exhibit substantially no toxicity, or substantially reduced toxicity, be immunogenic, and be functional as a vaccine.

11. Protein fragments for testing are easily generated through such means as recombinant expression techniques, using the sequences disclosed in the '934 application. Thus, it would have been routine, as of March 2, 1992, to generate fragments of *H. pylori* VacA protein and fragments of cytotoxin associated immunodominant antigen (CagA)[2]. Such fragments could then be used for further determinations of toxicity, immunogenicity, and vaccine efficacy.

12. Regarding toxicity, *in vitro* vacuolation assays can be used for the routine determination of toxic and non-toxic VacA protein, or fragments thereof. Exhibit B is a copy of Reyrat *et al.*, 1999, *Mol. Microbiol* 34:197-204, which is a review of the structure and activity of VacA. VacA was first described to induce vacuolation (the formation of large vacuoles) of mammalian cells *in vitro*, in 1988 (*see* page 199, column 2 of Exhibit B). Fig. 1A of Exhibit B shows a microscopic view of the vacuolating activity of purified VacA protein on HeLa cells. Thus, at the time of filing, those skilled in the art could have used an *in vitro* vacuolation assay on mammalian cells to routinely distinguish toxic and non-toxic VacA fragments.

13. Animal models used for the study of *H. pylori* infection were known prior to March 2, 1992. Such animal models of *H. pylori* infection include the gnotobiotic piglet (Krakowka *et al.*, 1987, Infect. Immun. 55:2789-2796) (Exhibit C) and the gnotobiotic dog (Radin *et al.*, 1990, Infect. Immun. 58:2606-2612) (Exhibit D). Animal models present convenient *in vivo* assay systems for routinely distinguishing non-toxic VacA protein, or fragments thereof, from those which exhibit toxicity. For example, Telford *et al.*, 1994, *J. Exp. Med.* 179:1653-1658 (Exhibit E), describes an *in vivo* mouse model of *H. pylori*-induced gastric ulceration, used to show that the VacA protein is responsible for the epithelial erosion seen in *H. pylori* infection. Sonicated, VacA-producing *H.*

[2]In the specification of the '934 application, *H. pylori* cytotoxin associated immunodominant antigen is referred to as "CAI." However, the current terminology used for this protein is the "cytotoxin-associated gene A" or "CagA" antigen.

pylori cells were shown to induce erosive lesions of the gastric mucosa, when orally administered to mice (Figure 1 (b and c)). Administration of purified VacA to mice also resulted in extensive tissue damage and mucosal erosion (Figure 1 (d, e, and f)). Thus, *H. pylori* infection models can be used, and could have been used at the time of filing, for routinely distinguishing toxic and non-toxic VacA fragments.

14. Regarding immunogenicity, one of skill in the art could have employed classical immunological assays to screen for antibody production in response to immunizations with fragments of *H. pylori* cytotoxin protein. These include, for example, 1) enzyme-linked immunosorbent assay (ELISA), 2) proliferation assays of cells from lymphoid organs, and 3) evaluation of the number of cells producing antibodies to a given antigen. Detailed protocols for these standard assays can be found in any manual on immunology. The Handbook of Experimental Immunology, Weir & Blackwell (eds.), 1986, which is cited at page 5, lines 22 - 24 of the specification, is a good example of such a manual, available to those of skill in the art at the time of filing of the application. Current Protocols in Immunology, John Wiley & Sons, New York, NY, which has been published since 1991, is another example of such a manual available to those of skill in the art. Thus, it would have been routine to determine which fragments of cytotoxin protein would generate an immune response, at the time of filing of the '934 application.

15. Regarding vaccine function, at page 15, lines 14 - 17, the specification of the '934 application defines a vaccine as "capable of eliciting protection against *H. pylori*." Furthermore, the vaccines of the invention can be prophylactic, therapeutic, or both (*see* page 38, line 39 - page 40, line 2, of the '934 application). Demonstration of a prophylactic or therapeutic effect of a protein, or polypeptide fragment of a protein, could have been carried out using routine functional experiments and assays. Functional experiments include the administration of a candidate vaccine to animals susceptible to *H. pylori* infection, either before challenge with the pathogen (prophylaxis

determination) or after infection has taken place (treatment determination). Animal models of disease provide convenient environments for such vaccine testing. *See* ¶ 13 above.

16. Exhibit F is a copy of Nedrud, 1999, *FEMS Immunol. Med. Microbiol.* 24:243-50, which is a review of animal models of *H. pylori* infection that have been established, including the pig, dog, gerbil, monkey, and ferret. Such models have been used since 1987 to examine infection-related disease processes and evaluate vaccines, and they can be used routinely to determine the effectiveness of *H. pylori* proteins and polypeptide fragments as vaccines against the infection.

17. Exhibit G is a copy of Ghiara *et al.*, 1997, *Infect. Immun.* 65:4996-5002, of which I am a co-author, and which shows that therapeutic vaccination with full-length recombinant *H. pylori* proteins, including VacA, can eradicate chronic *H. pylori* infection in a mouse model, and protect against subsequent challenge. Figure 3 of Exhibit G presents the results of therapeutic vaccination. Vaccination with full-length recombinant VacA protein (indicated as "Tox100") resolved the infection in about 92% of the mice. Full-length recombinant CagA protein yielded a 70% eradication of infection rate. Furthermore, once therapeutically treated, the mice are also protected from further challenge with *H. pylori*. Figure 5 presents the results of a study of reinfection rate, and shows that therapeutic vaccination with the recombinant VacA protein protected 70% of the mice from reinfection with *H. pylori*.

18. In the Action, the Examiner also asserted that a mucosal adjuvant is required for effective *H. pylori* component vaccines. I respectfully disagree with this characterization of the state of the art. Exhibit H is a copy of published PCT application PCT/IB99/00851, of which I am a co-inventor, and which teaches that mucosal delivery and mucosal adjuvants are <u>not</u> required for effective *H. pylori* component vaccines. This PCT application presents the results of intramuscular immunization studies with *H. pylori* component vaccines, in a dog model. Page 21 presents the

protocol for immunization. Full-length recombinant *H. pylori* proteins -- VacA, CagA, and neutrophil activating protein (NAP) -- were used as vaccine components for intramuscular immunizations. The adjuvant was aluminum hydroxide, *i.e.*, not a mucosal adjuvant.

19. Exhibit H demonstrates that the intramuscular immunizations induced high serum titers of antigen-specific antibodies to each of the *H. pylori* component proteins in the vaccine (*see* Figure 5A (VacA), Figure 5B (CagA), and Figure 5C (NAP)). Furthermore, intramuscular immunization was effective to protect all of the dogs from challenge with *H. pylori*. No symptoms of *H. pylori* infection were evident in the intramuscularly vaccinated dogs (page 12, lines 24 - 25). At 10 and at 42 days post-challenge, the intramuscularly vaccinated dogs' antral biopsies and gastric lavages were negative for urease[3] activity (page 12, lines 26 - 29, and page 14, Table 2). Furthermore, at 42 days post-challenge, intramuscularly vaccinated dogs had normal mucosa, without the signs of hyperemia or edema seen in the *H. pylori*-infected control animals (page 14, lines 4 - 10, and Figures 1A and 1B).

20. In the Action, the Examiner also rejected the claims as anticipated by Cover *et al.* (1992). I respectfully disagree. Cover *et al.* (1992) describes purified, native *H. pylori* 87kDa cytotoxin protein and reports that it is toxic. Cover *et al.* (1992) also describes the immunization of a rabbit with SDS-PAGE purified, denatured cytotoxin to generate an antisera, containing antibodies specific for the 87 kDa cytotoxin protein (as shown by immunoblot), and capable of neutralizing native cytotoxin vacuolating activity.

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[3]Urease is another *H. pylori* protein (*see* '934 application page 2, lines 8 - 14). A urease activity assay (*see* '934 application page 46, lines 14 - 16) is a means of detecting the presence of *H. pylori* infection, both in the experimental and the clinical setting.

21. The claims of the '934 application are directed to (1) recombinantly produced *H. pylori* VacA polypeptides, exhibiting substantially no toxicity, or substantially reduced toxicity, (2) vaccines comprising VacA polypeptides of (1), exhibiting substantially no toxicity, or substantially reduced toxicity, and (3) methods of making these vaccines. Manetti *et al.* (1995) (Exhibit A) shows a recombinant VacA protein that is non-toxic. The results show that the recombinant VacA protein, and two recombinant fragments of VacA protein, are non-toxic, having no vacuolating activity *in vitro* (page 4478, column 1). Although Manetti *et al.* (1995) (Exhibit A) reports that antibodies made against the denatured recombinant protein were unable to block the *in vitro* vacuolating activity of purified, native VacA (page 4478, Figure 4), Ghiara *et al.* (1997) (Exhibit G) disclose that the recombinant VacA is, nevertheless, capable of generating a protective effect against *H. pylori* infection.

22. Cover *et al.* (1992) does not disclose a recombinantly produced *H. pylori* VacA polypeptide exhibiting substantially no toxicity, or substantially reduced toxicity.

23. I declare that all statements made herein are of my own knowledge true and statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Giuseppe Del Giudice, M.D. Date